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Original article (Orijinal araştırma)

Morphometric analysis of wild-caught flies of *Drosophila* (Diptera: Drosophilidae) species: Altitudinal pattern of body size traits, wing morphology and sexual dimorphism

Doğadan yakalanan *Drosophila* (Diptera: Drosophilidae) türlerinin morfometrik analizi: Vücut büyüklüğü özelliklerinin yüksekliğe bağlı değişimi, kanat morfolojisi ve eşeysel dimorfizmi

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Summary

Literature concerning phenotypic variation among wild-caught drosophilids inhabiting varied ecological habitats is relatively rare. The present study explores pattern of body size traits along altitudinal gradients, and compensation to colder environments and reduced air pressure via adjustment of wing morphology at higher altitudes. Wild adult flies were collected in two extensive surveys during September-October 2014 and April-May 2015. All traits were measured for both the sexes to obtain data on sexual dimorphism. It was found that though these populations differed significantly in their size, as already known, they deviated from the expected reaction norms of size increase along altitudinal gradients as observed in several previous studies. This deviation from normal clinal trend can be attributed to variation in growth rates and development times at different altitudes which has important implications in overall reproductive success. Also, a significant increase in wing area of flies at higher altitude was recorded with dramatically lower wing loadings than flies that developed in comparatively warmer habitats, giving them an aerodynamic advantage at cold temperatures. Thorax width was also analyzed, possibly for the first time in wild-caught flies of Indian populations, revealing sexual dimorphism. The ratio of thorax length to width was greater than one for all species indicating that the thorax is more elongated in females, which may also influence the flight capacity of the sexes.

Keywords: Bergmann rule, Diptera, Drosophilidae, morphometric traits, plasticity

Özet

Çeşitli ekolojik habitatlarda yaşayan doğadan toplanmış Drosophila türleri arasındaki fenotipik çeşitlilik ile ilgili literatür sayısı nispeten azdır. Bu çalışmada vücut boyutu özelliklerinin yükseklik eğrileri boyunca olan uyumu ve daha yüksek yerlerde kanat morfolojisinin değişimiyle daha soğuk ortamlara ve daha düşük hava basıncına uyum sağlanması incelenmiştir. Doğadan ergin sinekler, Eylül-Ekim 2014 ve Nisan-Mayıs 2015 tarihlerinde iki kapsamlı sürvey ile toplanmıştır. Eşeysel dimorfizmi hakkında bilgi edinmek için her iki cinste de tüm özellikler ölçülmüştür. Bilindiği gibi, bu popülasyonların boyutlarında önemli farklılıklar olmasına rağmen, daha önceki birçok çalışmada gözlemlendiği gibi, yükseklik eğrileri boyunca boyut artışının beklenen reaksiyon normlarından sapmış oldukları bulunmuştur. Normal klinal eğimindeki bu sapma, genel üreme başarısında önemli etkileri olan farklı yüksekliklerde büyüme hızlarındaki ve gelişim zamanlarındaki farklılıklara bağlanabilir. Ayrıca, yüksek irtifadaki sineklerin kanat alanlarındaki önemli bir artış, karşılaştırmalı olarak daha sıcak habitatlarda gelişen sineklerden dramatik olarak çok daha düşük kanat yükleri ile rekor kırmış olmaları sayesinde onlara soğuk hava koşullarında aerodinamik bir avantaj sağlamıştır. Bu arada, muhtemelen Hint popülasyonlarının doğadan yakalanmış sineklerinde ilk kez, Thoraks genişliği, cinsel dimorfizmi açığa çıkararak analiz edilmiştir. Thoraks uzunluğunun genişliğe oranı tüm türler için birden fazla olup; bu da eşeylerin uçuş kapasitesini etkileyebilen thoraksın dişilerde daha uzun olduğunu göstermektedir.

Anahtar sözcükler: Bergmann kuralı, Diptera, Drosophilidae, morfometrik özellikler, plastisite

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Introduction

Analyzing variability of biological characteristics of organisms in response to some geographical gradient has been the prime approach to interpret macro-ecological patterns. The most widely cited studies are those of Bergmann, Allen, Gloger and Jordan (Huxley, 1942). Bergmann (1847) illustrated an eco-geographical pattern where organisms show increased body size or mass in colder climates, reflecting an altitudinal or latitudinal cline, with larger organisms at higher altitudes or latitudes. Ray (1960) first proposed the explanation that ectotherms might also follow Bergmann's rule as the average temperature decreases with increasing altitude or latitude and ectotherms reared at lower temperatures typically matured at larger sizes as compared to their conspecifics reared at higher temperatures. The related Allen's rule explained the significance of shorter limbs and less surface area in colder regions and vice versa (Daly, 1985). This has received little attention in insects (Ray, 1960; Peat et al., 2005) possibly because the results are complicated and conflict with Bergmann's rule. Some studies have argued Allen's rule as an exception rather than a rule, since protruding parts may be under strong selective pressures rather than other body parts related to thermoregulation (Stevenson, 1986).

The inverse Bergmann's rule has also been documented for insects (Van Voorhies, 1997; García-Barros, 2000) hinting that for diverse taxa body size even decreases from the tropics towards the poles, i.e., from warmer to colder climates (Mousseau, 1997). A species inhabiting different climatic conditions, adapts to the local climate often resulting in progressive genetic variations among populations. Also, the phenotypic plasticity, which is a general property of living beings, can contribute to geographical adaptation, if there is genetic variation for such plasticity (DeWitt & Scheiner, 2004). Phenotype along an environmental gradient is determined by its genotype. Though rigorous genetic studies should be conducted, a thorough understanding of geographic variation in morphology of ectotherms is prerequisite to compare the response curves to an environmental gradient of different populations, or the shape of the reaction norms. Understanding such adaptive capacity of natural populations and species has remained a central problem for evolutionary biologists, and comparative methods have long been powerful tools for exploring such capacities.

With thousands of described species, drosophilid flies appear as an irreplaceable model for investigating both phenotypic and genotypic adaptations. Biogeographically these species are usually classified either as tropical (cold sensitive) or temperate (cold tolerant). Only a few drosophilids can proliferate in both tropical and temperate environments and are termed widespread or often cosmopolitan (David & Tsacas, 1981; Powell, 1997). Geographical gradients as a proxy for climatic adaptations in such cosmopolitan flies have remained a fascinating arena for drosophilid researchers. Most studies have focused on latitudinal body size variations, in various species including *Drosophila robusta* Sturtevant, 1916 (Diptera: Drosophilidae) (Stalker & Carson, 1947), *Drosophila subobscura* Collin, 1936 (Prevosti, 1955), *Drosophila melanogaster* Meigen, 1830 and *Drosophila simulans* Sturtevant, 1919 (Capy et al., 1993; Gibert et al., 2004), *Drosophila kikkawai* Burla, 1954 (Karan et al., 1998) and *Zaprionus indianus* Gupta, 1970 (Karan et al., 2000; David et al., 2006a). Body size traits have been observed as highly-plastic showing increasing trend towards higher latitudes and colder places, and vice versa (Angilletta et al., 2004) often referred to as the temperature-size rule. Like the small size of wild flies (the expected result of natural selection) in warm tropical conditions, can be attributed to small genetic size due to the cline and smaller phenotypic size due to plasticity, favoring better fitness of small individuals in warm environments (Atkinson & Sibly, 1997; James et al., 1997).

However, no clinal pattern has been observed in some species (Loeschke et al., 2000), the temperature-size rule is not always convincing for traits such as thorax size (David et al., 2006a) and distinct phenotypes have been observed for distant geographic populations inhabiting the same thermal climatic conditions (Capy et al., 1993). Pitchers et al. (2013) studied variation in wing shape and size in *D. melanogaster* derived from populations at varying altitudes and latitudes across sub-Saharan Africa suggesting that selection responsible for these phenotypic clines may be more complex than just thermal adaptation. Klepsatel et al. (2014) also suggested that clinal patterns in morphology are not a simple function of changes in body size; instead, each trait might be subject to different selection pressures while Carreira et al. (2016) revealed weak clinal signals and a strong population effect on morphological variation and within-population genetic variation associated to the second chromosome. Singh (2015) has reviewed the work conducted on *Drosophila ananassae* Doleschall, 1858, *D. melanogaster*, *Drosophila nasuta* Lamb, 1914, *Drosophila bipectinata* Duda, 1923 and other species in India highlighting that these species vary in degree and pattern of genetic diversity and have evolved different mechanisms for adjusting to their environments. Evidently, such range of quantitative variation observed among geographic populations, call for deeper and more accurate investigations on these paradigmatic drosophilid species.

With most investigations along latitudes emphasizing the role of temperature in shaping different morphological traits, altitudinal gradient provides more rapid change in environmental conditions occurring over relatively small distance compared to equivalent distances over latitude. The Himalayan range is among the most intricate and diverse mountain systems in the world. It forms distinct geological and ecological entity, influencing climate and biotic aspects of the region. The varying topography promotes environmental heterogeneity at both temporal and spatial scales affecting diversity and distribution patterns of biodiversity elements. Uttarakhand State located in Central Himalayan region of India encompasses highly varied tropical to temperate like regimes in span of just few hundred kilometers due to its variable altitudinal terrain. Extensive explorations over the past decade identified more than 90 species from this region (Sarswat et al., 2015), with a significant number of novel species. Prior to this around 300 drosophilid species had been recorded throughout varied eco-geographical zones in India (Gupta, 2005; Kumar & Ajai, 2009). The change in environmental conditions occurring over short geographic distance in this Himalayan range profoundly effect the morphology, physiology and evolution of these flies.

The present study attempts to explore phenotypic variation among wild-caught drosophilids inhabiting varied ecological habitats, i.e., patterns of body size traits along altitudinal gradients (several traits were investigated along with different body shape indices) and compensation to colder environment and reduced air pressure via adjustment of wing morphology at higher altitudes (flight related traits such as wing length, wing width and wing area, along with wing aspect ratio and wing loading). All traits were measured for both the sexes to obtain data on sexual dimorphism. Thorax width was also analyzed, possibly for the first time in wild-caught flies of Indian populations, revealing difference between the sexes, with more elongated female thorax than male. In this study, it was found that though these populations differed significantly, they deviated from the expected, i.e., increasing size and shape related traits observed along altitudinal gradients in several previous studies.

Material and Methods

Sampling locations

Wild adult flies were collected in two surveys during September-October 2014 and April-May 2015, the most favorable months with optimum climatic conditions for proliferation of drosophilid population. Flies were collected by a range of sampling techniques along altitudinal transects starting from Srinagar-Garhwal (District-Pauri), Augustyamuni (District-Rudraprayag), Upper Chamoli (District-Chamoli), Mandal (District-Chamoli), Kanchula-Kharak (District-Chamoli) and Chopta (District-Rudraprayag). Data on weather conditions were obtained from local weather stations as well as the published climatological literature of the Indian Meteorological Department, Government of India (Table 1).

Table 1. Geographical locations and climatic conditions for different drosophilid populations analyzed in this study

	Geographical location			Climatic conditions			
Sampling station	Altitude (m)	Latitude (N)	Longitude (E)	T_{max} (°C)	T_{min} (°C)	T_{avg} (°C)	Annual precipitation (mm)
Srinagar-Garhwal	550	30° 22´	78° 78´	36.1	6.8	21.7	1371
Augustyamuni	800	30° 39´	79° 02´	34.7	6.5	20.7	1553
Upper Chamoli	1150	30° 24´	79° 21´	29.3	3.8	16.7	1305
Mandal	1600	30° 46´	79° 26´	28.8	3.6	16.4	1292
Kanchula-Kharak	2100	30° 49´	79° 22´	23.4	1.9	11.6	1445
Chopta	2700	30° 34´	79° 05´	20.8	-2.7	9.7	1626

The cosmopolitan or wide ranging species of fruit fly were collected from natural habitats employing range of techniques; the trap-bait method (small containers baited with yeasted banana or some other fermenting fruits, such as oranges, tomato, guava and apples, suspended on strings from the branches of bushes and trees), net sweeping (over natural feeding sites, such as decaying fruits and leaves, wild grasses and cultivated vegetation) and direct collection with aspirator (to trap flies directly while they were either courting or resting over the leaves, petals and fungi).

Identification and morphological study

Collected flies were etherized, categorized and subsequently identified through species specific morphological patterns common to both males and females according to Gupta (2005) and Markow & O'Grady, (2006), and online identification tools like BioCIS, JDD and FlyBase. For confirmation the detailed structures of male and female terminalia were observed under stereo microscope (Magnus MLX-DX model, at 10X magnification). The respective genital organs were detached from the adult body and cleared by warming in 10% KOH to around 100°C for 20-30 minute and observed in a droplet of glycerol. The morphological terminology, and the definitions of measurements and indices mostly followed McAlpine (1981), Zhang & Toda (1992) and Hu & Toda (2001). The examined specimens of all species were deposited in the Cytogenetics and Molecular Systematics Laboratory, Department of Zoology, HNB Garhwal University, Chauras Campus, Srinagar-Garhwal, Uttarakhand, India.

Measurement of morphometric traits

Twenty-five wild-caught flies of each sex per sampling location of five species viz., *Drosophila immigrans* Sturtevant, 1921, *Drosophila nepalensis* Okada, 1955, *Drosophila repleta* Wollaston, 1858, *Scaptomyza himalayana* Takada, 1970 and *Zaprionus grandis* Kikkawa & Peng, 1938 were measured for various morphometric traits related to head, thorax and wings, along with several body indices and flight traits. Major metric traits (related to size) analyzed were wing length (W) measured from the thoracic articulation to the tip of post-scutellum laterally, wing width (w) along the mid vertical line of the wing and thorax length (T) laterally from the neck to the tip of scutellum. Thorax width (t) was measured probably for the first time in wild-caught flies of Indian population, from a ventral view as the distance between the bases of the two major, posterior sternopleural bristles. Though much literature is available on wing and thorax length of several drosophilid species, thorax width has only been rarely reported.

An ocular micrometer was used for all measurements, and micrometer observations were transformed according to the magnifications and expressed in mm. Apart from these size related traits different ratios were also calculated. The W/T ratio, which describes the relative proportion of wing with respect to thorax, has been shown to have strong negative correlation with wing loading and provides information on flight capacity (David et al., 2006b). The elongation index, the ratio of thorax length to thorax width, increase with elongation of the thorax. The ratio of wing length to thorax width was also calculated. These ratios provide useful indices of the shape of drosophilid flies and have been considered as shape indices. All the morphometric studies were done in a temperature-controlled room set to 25ºC.

The standard methods widely reported in literature to calculate wing area, wing aspect ratio and wing-load index were followed to estimate flight related traits in this study (Stalker, 1980; Azevedo et al., 1998; Van't Land et al., 1999). Wing area (mm^2) was estimated as the product of wing length and wing width. Wing aspect ratio was measured as the ratio of wing length^2 to wing area. It is an important metric index which provides information about wing shape. Wing-load index was also calculated for the populations along altitudinal gradient, as the ratio of thorax volume to wing area. Two methods were followed in the cited studies for estimating wing loading, i.e., wing loading = body weight / wing area or thoracic volume / wing area. According to previous studies in wild-caught flies, variations in body weight due to age are difficult to control in females, however, such variations have been shown not to be significant in males. Accordingly, the age-related effects were nullified using thorax volume instead of body weight for wild-caught flies, as suggested by Stalker (1980). Thorax volume and body weight show positive linear correlation and thus it can be used to reduce uncontrolled variations in body weight due to age as well as nutrition. The thorax volume was calculated as the product of thorax length, thorax width and thorax depth.

All statistical analysis of the various traits was performed in IBM SPSS Statistics 20.0 software. Mean±SD values of 25 male and 25 female individuals per population were calculated for wild-caught drosophilid flies. ANOVAs were performed to examine the effects of the location altitude on the phenotypic traits. For almost all the quantitative traits, data on male and female individuals were treated separately. An attempt was also made to obtain data on sexual dimorphism for homologous traits that can be measured on both the sexes. Comparisons were made using the mean values of the females and males of wild-caught flies. Two methods have been published for estimating the extent of sexual dimorphism; difference between female and male trait values (F-M) and ratio of female to male trait values (F/M). Both measures were considered in previous studies (David et al., 2003; Huey et al., 2006) and the ratio method was considered to be preferable as it has no dimensionality and allows comparisons between different characters.

Results

Body size related traits

Body size related traits, in particular, are known to increase considerably with altitude and latitude as both genetics and temperature strongly mediate plasticity effects influencing these traits. In the present study, fly collection was done during the most favorable months of September-October and April-May (in 2014 and 2015, respectively), when the climatic conditions are optimum for proliferation of drosophilid population. Consistent with several earlier studies, an increasing trend for these traits in all species analyzed was also observed. There was a sharp increase in mean values up to Mandal (1600 m asl) and a significant decrease in values from Mandal to Chopta (2700 m asl). Further, the effect of altitude was also similar between the sexes, i.e., a similar trend of size variation with altitude was observed for both the sexes. Size variation was considerably marked across species. *Drosophila immigrans* had the maximum values for male body length and thorax length, while *Z. grandis* had the maximum mean value for female body length and thorax length, and maximum wing length for both males and females. The lowest values were observed for *S. himalayana* for body length and thorax length in both the sexes (Figure 1).

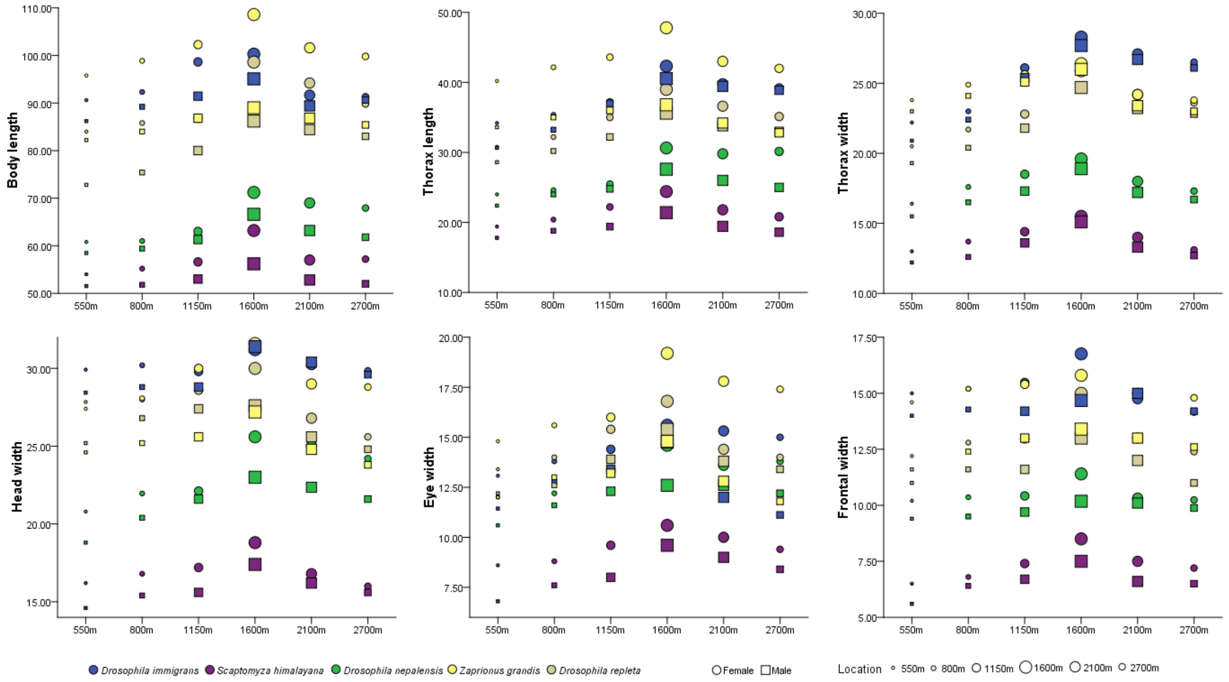

Figure 1. Variation in body size related traits along six sampling locations (symbol size is only indicative, representing trait measurements).

The variation in the traits with altitude was highly significant and there was also highly significant variation within and between groups for each trait sampled (Table 2). The shorter traits exhibited more variation because of a higher relative magnitude of measurement errors (Imasheva et al., 2000). Therefore, on average, males were more variable than females, and the thorax more variable than the wing length. Body length, and wing and thorax lengths varied significantly not only within species but also between species and also between sexes. The distributions for the sexes, however, overlapped considerably, such that males of some big species are much bigger than females of some small species.

Though these populations differed significantly along with altitude, they deviated from the expected increasing norm observed in other studies. The body size traits after quadratic transformation are presented in Figure 2. The analysis of the derivative curves reveals a fairly complex and sometimes biphasic shape, thus polynomial models are convenient for adjusting the response curve (David et al., 1997, 2004). A higher degree provides a better fit between the observations and the model; however, these are difficult to interpret biologically. There is, thus, a practical tradeoff between the need to increase the polynomial degree for a better fit and the use of a simple polynomial for an easier biological interpretation. The quadratic has obvious biological significance and may be called the characteristic values of the reaction norm (David et al., 1997).

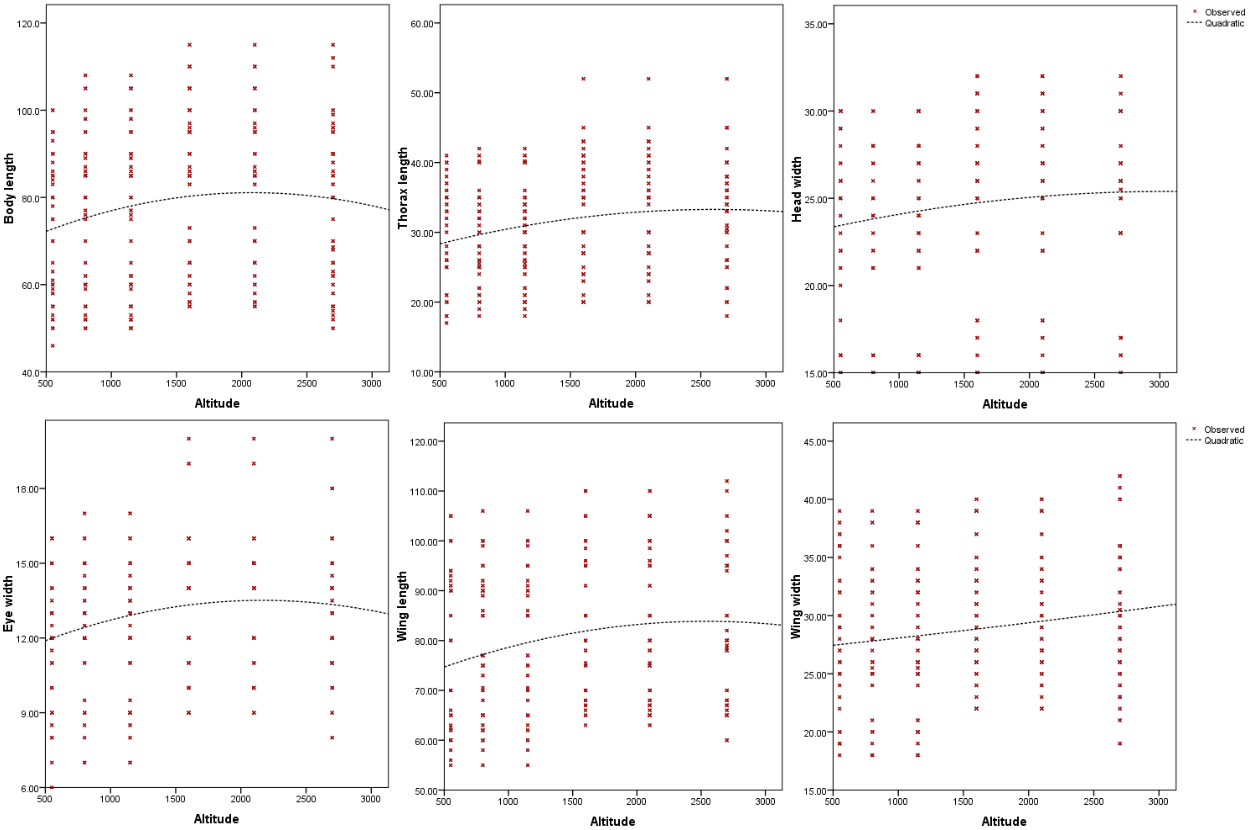

Figure 2. Results obtained after quadratic transformation of all trait reaction norms at the locations sampled.

Table 2. Variance of different traits explained by altitude as a factor (ANOVA) and post hoc analysis (Tukey HSD)

	Mean±SD						P value	Multiple comparisons P value						
	550m	800m	1150m	1600m	2100m	2700m								
									1	2	3	4	5	6
Body length	73.48±16.70	75.52±17.21	75.52±17.21	81.70±16.98	81.70±16.98	79.25±17.10	0.0087	1		0.076	0.076	0.00001	0.00001	0.002
								2	0.076		1	0.001	0.001	0.014
								3	0.076	1		0.001	0.001	0.014
								4	0.0001	0.001	0.001		1	0.059
								5	0.0001	0.001	0.001	1		0.059
								6	0.002	0.014	0.014	0.059	0.059	
Thorax length	29.60±7.23	29.09±6.28	29.09±6.29	33.48±7.73	33.48±7.73	32.85±7.97	0.0009	1		0.0971	0.0971	0.00001	0.00001	0.00009
								2	0.0971		1	0.00001	0.00001	0.00001
								3	0.0971	1		0.00001	0.00001	0.00001
								4	0.00001	0.00001	0.00001		1	0.0927
								5	0.00001	0.00001	0.00001	1		0.0927
								6	0.00009	0.00001	0.00001	0.0927	0.0927	
Head width	23.92±5.08	23.50±4.73	23.50±4.73	25.32±5.26	25.32±5.26	25.17±4.94	0.0025	1		0.0937	0.0937	0.022	0.022	0.060
								2	0.0937		1	0.001	0.001	0.003
								3	0.0937	1		0.001	0.001	0.003
								4	0.022	0.001	0.001		1	0.0999
								5	0.022	0.001	0.001	1		0.0999
								6	0.060	0.003	0.003	0.0999	0.0999	

Table 2. (Continued)

	Mean±SD						P value	Multiple comparisons P value Tukey HSD[#]						
	550m	800m	1150m	1600m	2100m	2700m								
Eye width	12.18±2.63	12.40±2.33	12.40±2.33	13.64±2.35	13.64±2.35	13.24±2.44	0.0019	1		0.0911	0.0911	0.00001	0.00001	0.00002
								2	0.0911		1	0.00001	0.00001	0.001
								3	0.0911	1		0.00001	0.00001	0.001
								4	0.00001	0.00001	0.00001		1	0.0430
								5	0.00001	0.00001	0.00001	1		0.0430
								6	0.00002	0.001	0.001	0.0430	0.0430	
Frontal width	11.35±3.19	10.30±2.58	10.30±2.58	11.24±3.18	11.24±3.18	11.36±2.89	0.0005	1		0.001	0.001	0.0998	0.0998	1
								2	0.001		1	0.005	0.005	0.001
								3	0.001	1		0.005	0.005	0.001
								4	0.0998	0.005	0.005		1	0.0998
								5	0.0998	0.005	0.005	1		0.0998
								6	1	0.001	0.001	0.0998	0.0998	
Wing length	75.95±16.18	77.15±13.73	77.15±13.73	83.68±14.78	83.68±14.78	83.43±14.20	0.00001	1		0.942	0.942	0.00001	0.00001	0.00001
								2	0.942		1	0.00001	0.00001	0.00002
								3	0.942	1		0.00001	0.00001	0.00002
								4	0.00001	0.00001	0.00001		1	1
								5	0.00001	0.00001	0.00001	1		1
								6	0.00001	0.00002	0.00002	1	1	
Wing width	28.38±6.37	27.11±5.70	27.11±5.70	29.82±5.40	29.82±5.40	30.07±6.10	0.0003	1		0.140	0.140	0.061	0.061	0.014
									0.140		1	0.00003	0.00003	0.00001
								3	0.140	1		0.00003	0.00003	0.00001
								4	0.061	0.00003		0.00003	1	0.997
								5	0.061	0.00003	0.00003		1	0.997
								6	0.014	0.00001	0.00001	0.997	0.997	

[#] In multiple comparisons Tukey HSD, numbers 1-6 represent the six sampling locations from lowest to highest.

Wing size and shape

Mean values of the wing size estimates showed an increasing trend, reaching the maximum at the highest altitude. Wing area was larger at higher altitudes and compensated for variation in body size (Figure 3). The relationship between wing and thorax lengths a useful parameter because it provides critical information on wing loading, wing beat frequency and presumably flight capacity. Maximum values for the ratio of wing length to thorax length were 3.77±0.05 in males and 3.55±0.07 in females, and for the ratio of wing length to thorax width were 5.28±0.08 in males and 5.63±0.09 in females observed at highest altitude, i.e., Chopta (2700 m asl) for *S. himalayana*. Owing to different shape and these variations in individuals their respective wing loads will not be exactly the same. Thorax width variability, a measure of elongation index (the ratio of thorax length to thorax width) was significantly higher in females indicating thorax is more elongated in female flies (Figure 4).

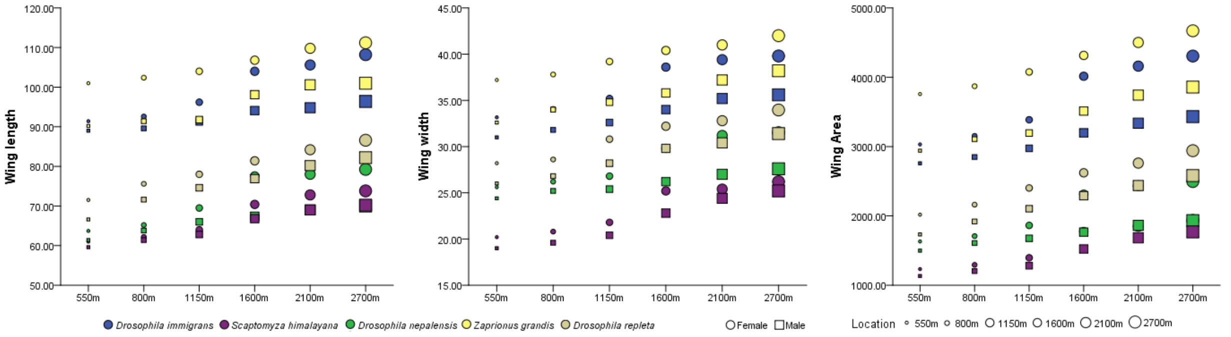

Figure 3. Wing length, wing width and wing area analyzed at six sampling locations (symbol size is only indicative, representing trait measurements).

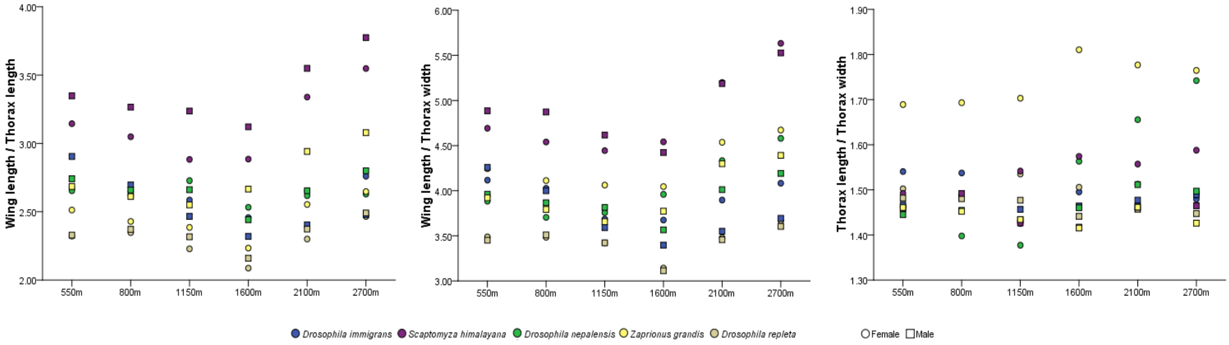

Figure 4. Variation in body size indices observed along the altitude gradient sampled.

Wing aspect ratio, an interpretation of the wing shape, also increased significantly from Mandal to Chopta, indicating elongation of wings at high altitude. The flies developing in cold habitats also had decreased wing loading compared to those developing at mid elevations with optimum temperature and longer growing season (Figure 5). Overall all traits, the mean square between subgroups was always superior to the mean square within subgroups and F was greater than unity. Such variations between populations are mainly due to long-range geographic variations. These were higher and statistically significant for almost all traits. This indicates that different traits do not always follow the same rules of variation exactly. Such a result gives evidence of parallel variation and suggests an adaptive significance.

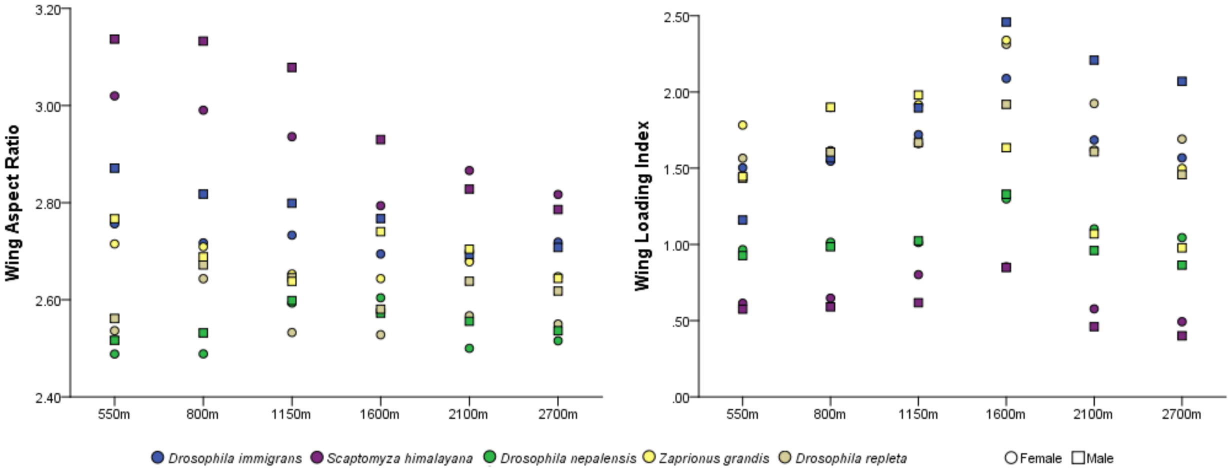

Figure 5. Flight performance indices: wing aspect ratio and wing loading for populations studied along an altitudinal transect.

Sexual dimorphism

The size related traits (e.g., body length, thorax length, thorax width, wing length and wing width) were much greater in females; however, a significant trend of increasing size with altitude was observed for both sexes. Two possible indices, the F to M ratio and the difference between F and M, provided the same information, however, the ratio was preferred as it is a non-dimensional measure not influenced by variations of the mean, thus allowing comparison between different traits. Values obtained at different altitudes were grouped to obtain a mean value of F to M ratio for a species (Table 3). For body length, and thorax and wing lengths, the F to M ratios were the highest, ranging up to 1.18, 1.24 and 1.11, respectively, all for *Z*. *grandis*. The ratio for thorax width, however, was much less with maximum value of 1.05 for *D*. *nepalensis, D*. *repleta* and *S*. *himalayana* indicating that males are more similar to females for this trait. The ratio of thorax length to thorax width, i.e., the elongation index, was greater than 1 obtained for all species, which indicates that the male thorax is more rounded than their female counterparts. A similar trend was observed for the wing length to thorax width ratio. The wing length to thorax length ratio was, however, very close to one in all five species, which can be attributed to the fact that these two traits have minimal dimorphism.

Discussion

The body size related traits vary both across and within species in response to the environment (e.g., nutrition, developmental temperature and stressors) as well as genetic factors. Our findings however, deviated from general temperature-size rules as the highest value for these traits were observed at mid elevation, hinting an adaptive interpretation that size is maximum under optimal physiological conditions. A similar trend was observed in both male and female flies. This deviation from normal clinal trend can be attributed to variation in growth rates and development times at different altitudes, which has important implications for overall reproductive success. It has been observed that some species show increasing size with altitude, although the reverse has also been seen (Chown & Klok, 2003), due to shortening of developmental time, in order to facilitate successful reproduction before the end of the season.

Table 3. Analysis of sexual dimorphism considering the F to M ratio with the percentage of variance obtained through ANOVA

	F/M Ratio, % Variance, P value														
	Drosophila immigrans			*Drosophila nepalensis*			*Drosophila repleta*			*Scaptomyza himalayana*			*Zaprionus grandis*		
Body length	1.042	17.66	*0.00091*	1.059	21.87	*0.00062*	1.120	33.21	*0.00098*	1.081	27.23	*0.00083*	1.180	37.19	*0.00099*
Thorax length	1.041	16.5	*0.06729*	1.098	26.72	*0.00091*	1.079	22.8	*0.00092*	1.117	29.12	*0.00075*	1.241	31.89	*0.00097*
Thorax width	1.028	26.8	*0.00072*	1.052	45.23	*0.00082*	1.050	39.17	*0.00075*	1.054	46.77	*0.00094*	1.029	27.56	*0.00091*
Wing length	1.076	19.1	*0.00088*	1.088	19.23	*0.00092*	1.056	16.82	*0.0008*	1.036	15.69	*0.00092*	1.109	21.87	*0.00083*
Head width	1.022	11.28	*0.05916*	1.093	22.13	*0.00083*	1.060	18.5	*0.00099*	1.074	19.21	*0.00082*	1.157	26.88	*0.00072*
Eye width	1.164	26.56	*0.00081*	1.107	22.13	*0.00099*	1.083	18.75	*0.00096*	1.159	24.6	*0.00082*	1.301	37.92	*0.00096*
Frontal width	1.058	13.78	*0.00081*	1.071	15.5	*0.00072*	1.116	18.7	*0.00084*	1.118	18.9	*0.00081*	1.196	24.51	*0.00084*
W/T#	1.036	11.23	*0.00082*	0.992	9.6	*0.07121*	0.979	8.77	*0.06111*	0.928	8.12	*0.05102*	0.895	6.63	*0.08121*
W/t#	1.047	21.32	*0.00092*	1.034	20.26	*0.00064*	1.006	18.45	*0.00093*	0.984	18.2	*0.00099*	1.078	24.56	*0.00062*
T/t#	1.012	19.56	*0.00073*	1.044	24.3	*0.00066*	1.028	22.95	*0.00091*	1.060	25.69	*0.00096*	1.207	37.98	*0.00077*

#W, wing length; T, thorax length; t, thorax width.

Initial increases in these traits observed in our data can generally be explained by a negative relationship between developmental temperature and size in a non-resource-limited environment up to mid altitude (T_{avg} = 16.5°C and ~1300 mm of annual precipitation at mid elevation, i.e., Mandal), having optimum habitat conditions, such as a warmer, but still cool, non-seasonal environment. Support for these observations has been given by several workers studying different insect genera (Smith et al., 2000). . Mid elevation peaks observed for most of the size related traits in our study also support the hypothesis that insect body size clines are influenced by the length of the insect's generation time relative to local season length (Mousseau, 1997; Dillon et al., 2006; Chown & Gaston, 2010). The insect populations living in the colder regions of the range of their species are more likely to experience significant resource limitations (T_{avg} = 11.6°C and ~1450 mm of annual precipitation at Kanchula-Kharak; T_{avg} 9.7°C and ~1600 mm of annual precipitation at Chopta) leading to size decrease as they must have more rapid development to complete a generation during the available growing season, or they are under risk of running out of optimum time or resources prior to completing development. The generation time can be shortened by either maturing to adult at a smaller body size or by increasing the growth rate. However, insects can increase their growth rate only to certain extent due to thermodynamic constraints (Gillooly et al., 2002).

These patterns in body size traits, although being supported by the Bergmann’s, Allen’s or temperature–size rules, include several instances showing a reverse or even the absence of pattern in body size clines along altitudinal gradients. The optimal size of a trait is thus a reflection of a trade-off between the costs and benefits of body size in a particular environment. As studied by Ray (1960), Bergmann’s and Allen’s rules apply to both endotherms and ectotherms but only at the intraspecific level (*sensu* Mayr 1942). Ray considered temperature as the only factor related to Allen’s rule and his results for *Drosophila* species followed both Bergmann’s and Allen’s rules as leg proportions with respect to body size decreased with decreasing temperature. In species showing inverse Bergmann’s pattern, the geographical variation in body size proportions of protruding parts may show differential allometric growth in different areas due to time constraints on development and growth imposed by abiotic factors that regulate season and time available for reproduction. Alternatively, it also reflects true Allenian variation related to thermoregulation (Bidau et al., 2012).

Other environmental factors challenging to insects are reduced mean temperatures and low barometric pressure at higher altitude. Wing morphology was also studied, as it has been shown that decreased temperature and air pressure severely compromises walking speed and flight performance. A significant increase in wing length and width was observed with altitude. Another factor for improved flight performance of flies at cold temperatures was a dramatic increase in wing area relative to body mass. Flies from cold environments have been shown to have improved flight performance from increased wing area relative to their body mass, which reduces induced power requirements and increases lift production (Dudley, 2000). Wing aspect ratio, an interpretation of the wing shape, also increased significantly from Mandal to Chopta, indicating elongation of wings at high altitude. Changes in wing shape may also improve flight performance. Elongation of wings, while maintaining the wing area improves some aspects of flight performance as higher, translational velocity of wing tips at same angular velocity produces greater aerodynamic forces (Frazier et al., 2008). With a significant increase in wing area, flies at higher altitude had dramatically lower wing loadings than flies that developed in comparatively warmer habitats, giving them an aerodynamic advantage at cold temperatures, as has been observed in several other studies (Gilchrist & Huey, 2004; Frazier et al., 2008). Theoretically reduced wing loading is advantageous for generating lift during flight via increasing mechanical power output, thus a compensatory mechanism for improved flight performance (Norry et al., 2001).

Though several studies focus on altitudinal variation in overall body size, most of them ignore sexual size dimorphism. Here, we assessed female to male ratio depicting that the size variations between the sexes were significant for different traits. It indicates that some or the other environmental/ecological factor varying with altitude is differentially affecting selection on the sexes. Also, though a similar trend in size traits with altitude was observed for both the sexes, females were larger than males, a pattern that is consistent with the general female-biased dimorphism observed in most species of insects (Fairbairn, 1997). It has also been suggested that variation in sexual size dimorphism among populations is due to canalization of traits closely associated with fitness, thus the F to M ratios for body length, and thorax and wing lengths, were much higher indicating these traits are more important to fitness in females thus were developmentally canalized in response to environmental perturbations (Fairbairn, 2005) as compared to thorax width for which the ratio was smaller indicating little influence of this trait in female or male fitness. The different optima of these traits for each sex might also be a consequence of different ecological or social roles as suggested under dimorphic niche hypothesis (Slatkin, 1984), i.e., if there are intrinsic differences between sexes for their energetic needs to ensure successful reproduction, then it is likely that different optima exist for each sex. This could be reasonable a explanation, as reproductive capacity of most species is limited by the size of females, and adult female size is probably dependent, to an extent, on limited ecological resources, as along an altitudinal gradient.

Further, three ratios were calculated from three size-related traits. The higher value obtained for thorax length to thorax width ratio, i.e., the elongation index, indicate that the thorax is more elongated in the females, which may significantly influence the flight capacity between sexes. The second ratio for the wing length to thorax width showed similar trend, however its biological meaning is less obvious. The wing length to thorax length ratio characterizes the relative proportion of the wing with respect to the thorax. As established in previous studies (Petavy et al., 1997; David et al., 2006b), this ratio is strongly negatively correlated with wing loading, and provides information on flight capacity thus a value closer to unity in all five species hints that flight compensatory adjustments via wing loading are similar among species as well as sexes.

It is concluded that the general pattern of body size related traits along altitudinal gradient cannot be well predicted by any single eco geographic rule. The correlation between any environmental gradient to body size is much more complex and depends upon its life history and several environmental aspects. As the populations facing greater time constraint for development and reproduction are likely to mature at smaller body size at high altitudes (contrary to Bergmann). Notably, some initial reports of this “inverted” empirical body size pattern seem to support Allen’s rule (Ruibal, 1955; Martof & Humphries, 1959; Ray 1960). Also, some studies suggest that beneficial plasticity or acclimation may contribute to the ability of *Drosophila* species to occupy varied habitats while others have shown it may not be an evolutionarily important mechanism. As for wing-loading, phenotypic plasticity may be more important than population level genetic differences at higher altitude. It can be assumed that there is strong developmental plasticity of flight performance due to decreased wing loading in response to cold developmental temperatures at higher elevations. Developmental plasticity and/or acclimatization thus may be an imperative phenomenon, especially when local genetic adaptation may be hindered by high gene flow in mobile insects occupying large geographic ranges as drosophilids.

The future studies in this region could explore, the relative contributions of size variation and growth rate to early development, to determine the evolutionary constraints on these important fitness related variables. Also, the significant values obtained for sexual dimorphism of almost all body size related traits suggests that these traits share a common genetic basis. Investigating how the sex-determining genetic cascade interferes with various quantitative traits would also be worthwhile.

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References

Angilletta, M. J., T. S. Steury & M. W. Sears, 2004. Temperature growth rate and body size in ectotherms: Fitting pieces of a life history puzzle. Integrative and Comparative Biology, 44: 498-509.

Atkinson, D. & R. M. Sibly, 1997. Why are organisms usually bigger in colder environments? Making sense of a life history puzzle. Trends in Ecology and Evolution, 12: 235-239.

Azevedo, R. B. R., A. C. James, J. McCabe & L. Partridge, 1998. Latitudinal variation of wing: Thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. Evolution, 52: 1353-1362.

Bergmann, C., 1847. Uber die Verh Altnisse der WArme Okonomie der Thiere zu ihrer Grosse. Gottinger Studien, 3: 595-708.

Bidau, C. J., C. I. Miño, E. R. Castillo & D. A. Martí, 2012. Effects of abiotic factors on the geographic distribution of body size variation and chromosomal polymorphisms in two neotropical grasshopper species (Dichroplus: Melanoplinae: Acrididae). Psyche: A Journal of Entomology, 2012: 1-11.

Capy, P., E. Pla & J. R. David, 1993. Phenotypic and genetic variability of morphometrical traits in natural populations of *Drosophila melanogaster* and *D. simulans* I Geographic variations. Genetics Selection Evolution, 25: 517-536.

Carreira, V. P., J. Mensch, E. Hasson & J. J. Fanara, 2016. Natural genetic variation and candidate genes for morphological traits in *Drosophila melanogaster*. PLoS ONE, 11 (7): e0160069.

Chown, S. L. & C. J. Klok, 2003. Altitudinal body size clines: Latitudinal effects associated with changing seasonality. Ecography, 26: 445-455.

Chown, S. L. & K. J. Gaston, 2010. Body size variation in insects: A macro-ecological perspective. Biological Reviews, 85: 139-169.

Daly, H. V., 1985. Insect morphometrics. Annual Reviews of Entomology, 30: 415-438.

David, J. R., L. O. Araripe, B. C. Bitner-Mathe, P. Capy, B. Goni, L. B. Klaczko, H. Legout, M. B. Martins, J. Vouidibio, A. Yassin & B. Moreteau, 2006a. Quantitative trait analysis and geographic variability of natural populations of *Zaprionus indianus*, a recent invader in Brazil. Heredity, 96: 53-62.

David, J. R., P. Gibert, E. Gravot, G. Petavy, J. P. Morin, D. Karan & B. Moreteau, 1997. Phenotypic plasticity and developmental temperature in *Drosophila*: Analysis and significance of reaction norms of morphometrical traits. Journal of Thermal Biology, 22: 441-451.

David, J. R., P. Gibert, S. Mignon-Grasteau, H. Legout, G. Petavy, C. Beaumont & B. Moreteau, 2003. Genetic variability of sexual sex dimorphism in a natural population of *Drosophila melanogaster*: An isofemale line approach. Journal of Genetics, 82: 101-110.

David, J. R., P. Gibert & B. Moreteau, 2004. “Evolution of Reaction Norms, 50-63”. In: Phenotypic Plasticity-Functional and Conceptual Approaches (Eds. T. J. DeWitt & S. M. Scheiner). Oxford University Press, 272 pp.

David, J. R., H. Legout & B. Moreteau, 2006b. Phenotypic plasticity of body size in a temperate population of *Drosophila melanogaster*: When the temperature-size rule does not apply. Journal of Genetics, 85: 9-23.

David, J. R. & L. Tsacas, 1981. Cosmopolitan subcosmopolitan and widespread species: Different strategies within the Drosophilid family (Diptera). CR Society of Biogeography, 57: 11-26.

DeWitt, T. J. & S. M. Scheiner, 2004. Phenotypic Plasticity-Functional and Conceptual Approaches. Oxford University Press, Oxford, 272 pp.

Dillon, M., M. Frazier & R. Dudley, 2006. In to thin air-Physiology and evolution of alpine insects. Integrative and Comparative Biology, 46 (1): 49-61.

Dudley, R., 2000. The Biomechanics of Insect Flight-Form, Function, Evolution. Princeton University Press, Princeton, 496 pp.

Fairbairn, D. J, 1997. Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. Annual Review of Ecology and Systematics, 28: 659-687.

Fairbairn, D. J, 2005. Allometry for sexual size dimorphism: testing two hypotheses for Rensch’s rule in the water strider *Aquarius remigis*. American Naturalist, 166: 69-84.

Frazier, M. R., J. F. Harrison, S. D. Kirkton & S. P. Roberts, 2008. Cold rearing improves cold-flight performance in *Drosophila* via changes in wing morphology. Journal of Experimental Biology, 211: 2116-2122.

García-Barros, E., 2000. Climaytamaño en mariposas diurnas (Lepidoptera: Papilionidae). Boletín de la Asociación Española de Entomología, 24: 47-64.

Gibert, P., P. Capy, A. Imasheva, B. Moreteau, J. P. Morin, G. Petavy & J. R. David, 2004. Comparative analysis of morphological traits among *Drosophila melanogaster* and *D. simulans*: Genetic variability, clines and phenotypic plasticity. Genetica, 120: 165-179.

Gilchrist, G. W. & R. B. Huey, 2004. Plastic and genetic variation in wing loading as a function of temperature within and among parallel clines in *Drosophila subobscura*. Integrative and Comparative Biology, 44: 461-470.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage & J. H. Brown, 2002. Effects of size and temperature on developmental time. Nature, 417: 70-73.

Gupta, J. P., 2005. A monograph on Indian Drosophilidae. Journal of Scientific Research, 5: 1-252.

Hu, Y. G. & M. J. Toda, 2001. Polyphyly of *Lordiphosa* and its relationships in Drosophilinae (Diptera: Drosophilidae). Systematic Entomology, 26: 15-31.

Huey, R. B., B. Moreteau, J. C. Moreteau, P. Gibert, G. W. Gilchrist, A. R. Ives, T. Garland Jr & J. R. David, 2006. Sexual size dimorphism in a *Drosophila* clade, the *D. obscura* group. Zoology, 109: 318-330.

Huxley, J., 1942. Evolution: The Modern Synthesis. Allen and Unwin Limited, London, 645 pp.

Imasheva, A. G., B. Moreteau & J. R. David, 2000. Growth temperature and genetic variability of wing dimensions in *Drosophila*: Opposite trends in two sibling species. Genetics Research, 76: 237-247.

James, A. C., R. B. R. Azevedo & L. Partridge, 1997. Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. Genetics, 146: 881-890.

Karan, D., A. K. Munjal, P. Gibert, B. Moreteau, R. Parkash & J. R. David, 1998. Latitudinal clines for morphometrical traits in *Drosophila kikkawai*: A study of natural populations from the Indian subcontinent. Genetics Research, 71: 31-38.

Karan, D., S. Dubey, B. Moreteau, R. Parkash & J. R. David, 2000. Geographical clines for quantitative traits in natural populations of a tropical drosophilid: *Zaprionus indianus*. Genetica, 108: 91-100.

Klepsatel, P., M. Gáliková, C. D. Huber & T. Flatt, 2014. Similarities and differences in altitudinal versus latitudinal variation for morphological traits in *Drosophila melanogaster*. Evolution, 68 (5): 1385-98.

Kumar, R. & K. Ajai, 2009. Studies on Drosophilidae (Diptera: Drosophilidae) of Gujarat state in India. *Drosophila* Information Services, 92: 106.

Loeschke, V., J. Bundgaard & J. S. F. Barker, 2000. Variation in body size and life history traits in *Drosophila aldrichi* and *D. buzzatii* from a latitudinal cline in eastern Australia. Heredity, 85: 423-433.

Markow, T. & P. M. O'Grady, 2006. *Drosophila*-A Guide to Species Identification and Use. London Academic Press, London, 250 pp.

Martof, B. S. & R. L. Humphries, 1959. Geographic variation in the wood frog, *Rana sylvatica*. The American Midland Naturalist, 6: 350-389.

Mayr, E., 1942. Systematics and the Origin of Species. Columbia University Press, New York, 382 pp.

McAlpine, J. F., 1981. "Morphology and Terminology-Adults, 9-63". In: Manual of Nearctic Diptera, Volume 1, Agriculture Canada Monograph No. 27 (Eds. J. F. McAlpine, B. V. Peterson, G. E. Shewell, H. J. Teskey, J. R. Vockeroth & D. M. Wood). Ontario Biosystematics Research Institute, Research Branch, Supply & Services Canada, Hull, Quebec, Ottawa Canada, 674 pp.

Mousseau, T. A., 1997. Ectotherms follow the converse to Bergmann's rule. Evolution, 51: 630-632.

Norry, F., O. Bubliy & V. Loeschcke, 2001. Developmental time, body size and wing loading in *Drosophila buzzatii* from lowland and highland populations in Argentina. Hereditas, 135 (1): 35-40.

Peat, J., B. Darvill, J. Ellis & D. Goulson, 2005. Effects of climate on intra- and interspecific size variation in bumble-bees. Functional Ecology, 19 (1): 145-151.

Petavy G., J. P. Morin, B. Moreteau & J. R. David, 1997 Growth temperature and phenotypic plasticity in two *Drosophila* sibling species: probable adaptive changes in flight capacities. Journal of Evolutionary Biology, 10: 875–887.

Pitchers, W., J. E. Pool & I. Dworkin, 2013. Altitudinal clinal variation in wing size and shape in African *Drosophila melanogaster*: One cline or many? Evolution, 67 (2): 438-452.

Powell, J. R., 1997. Progress and Prospects in Evolutionary Biology- The *Drosophila* Model. Oxford University Press, Oxford, 562 pp.

Prevosti, A., 1955. Geographical variability in quantitative traits in populations of *Drosophila* subobscura. Cold Spring Harbor Symposium Quantitative Biology, 20: 294-299.

Ray, C., 1960. The application of Bergmann's and Allen's rules to poikilotherms. Journal of Morphology, 106: 85-108.

Ruibal, R., 1955. A study of altitudinal races in *Rana pipiens*. Evolution, 9: 322-338.

Sarswat, M., R. S. Fartyal, P. C. Sati, M. Kandpal & B. K. Singh, 2015. Diversity and distribution pattern of drosophilids (Diptera, Drosophilidae) along an altitudinal transect in Uttarakhand and its implication in cytogenetics & molecular systematics study. Journal of Entomological Research, 39 (4): 311-322.

Singh, B. N., 2015. Species and genetic diversity in the genus *Drosophila* inhabiting the Indian subcontinent. Journal of Genetics, 94: 351-361.

Slatkin, M., 1984. Ecological causes of sexual dimorphism. Evolution, 38: 622-630.

Smith, R. J., A. Hines, S. Richmond, M. Merrick, A. Drew & R. Fargo, 2000. Altitudinal variation in body size and population density of *Nicrophorus investigator* (Coleoptera: Silphidae). Environmental Entomology, 29: 290-298.

Stalker, H. D., 1980. Chromosome studies in wild populations of *Drosophila melanogaster*. II. Relationship of inversion frequencies to latitude, season, wing-loading and flight activity. Genetics, 95: 211-223.

Stalker, H. D. & H. L. Carson, 1947. Morphological variation in natural populations of *Drosophila robusta* Sturtevant. Evolution, 1: 237-248.

Stevenson, R. D., 1986. Allen's rule in North American rabbits (*Sylvilagus*) and hares (*Lepus*) is an exception, not a rule. Journal of Mammalogy, 67: 312-316.

Van Voorhies, W. A., 1997. On the adaptive nature of Bergmann size clines: A reply to Mousseau, Partridge and Coyne. Evolution, 51: 635-640.

Van't Land, J., B. J. Zwaan, W. F. Van Putten, A. Kamping & W. Van Delden, 1999. Latitudinal variation in wild populations of *Drosophila melanogaster*: Heritabilities and reaction norms. Journal of Evolutionary Biology, 12: 222-232.

Zhang, W. & M. J. Toda, 1992. A new species-subgroup of the *Drosophila immigrans* species-group (Diptera, Drosophilidae), with description of two new species from China and revision of taxonomic terminology. Japanese Journal of Entomology, 60: 839-850.